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Short communication

Direct analysis of ethylene glycol in human serum on the basis of analyte adduct formation and liquid chromatography–tandem mass spectrometry

Marek Dziadosz

Institute of Legal Medicine, Hannover Medical School (MHH), Carl-Neuberg-Str. 1, D-30625 Hannover, Germany

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ABSTRACT

The aim of this work was to develop a fast, cost-effective and time-saving liquid chromatography-tandem mass spectrometry (LC–MS/MS) analytical method for the analysis of ethylene glycol (EG) in human serum. For these purposes, the formation/fragmentation of an EG adduct ion with sodium and sodium acetate was applied in the positive electrospray mode for signal detection. Adduct identification was performed with appropriate infusion experiments based on analyte solutions prepared in different concentrations. Corresponding analyte adduct ions and adduct ion fragments could be identified both for EG and the deuterated internal standard (EG-D4). Protein precipitation was used as sample preparation. The analysis of the supernatant was performed with a Luna 5 µm C18 (2) 100 A, 150 mm × 2 mm analytical column and a mobile phase consisting of 95% A (H_2O/methanol = 95/5, v/v) and 5% B (H_2O/methanol = 3/97, v/v), both with 10 mmol L^{-1} ammonium acetate and 0.1% acetic acid. Method linearity was examined in the range of 100–4000 µg/mL and the calculated limit of detection/quantification was 35/98 µg/mL. However, on the basis of the signal to noise ratio, quantification was recommended at a limit of 300 µg/mL. Additionally, the examined precision, accuracy, stability, selectivity and matrix effect demonstrated that the method is a practicable alternative for EG quantification in human serum. In comparison to other methods based on liquid chromatography, the strategy presented made for the first time the EG analysis without analyte derivatisation possible.

1. Introduction

Ethylene glycol (EG) is a colorless, odorless, toxic polyhydroxylic alcohol with a sweet taste and a molecular mass of 62 Da. It is also a popular ingredient of many automotive antifreeze liquids. Its physical properties and soporific effects (similar to ethanol) are responsible for its accidental or suicidal use as beverage, which have led to many intoxications in the past [1]. A fast identification of EG intoxication by quantification in human serum is the basis for the application of different therapeutic strategies, which can prevent organ damage and death. Therefore, appropriate analytical methods have to be applied in toxicological/clinical laboratories.

Different analytical strategies applied for EG analysis with gas and liquid chromatography were already published [2–9]. Since both analytical techniques usually need an appropriate EG derivatisation procedure for analyte detection, a multi-step sample preparation is needed. When the combination of gas chromatography and the flame ionisation detector was applied, EG could also be analysed by direct injection [10,11]. However, in methods without mass spectrometric detection the risk for misinterpretation of interfering peaks is a common issue. Therefore, these methods are not favored in the routine. The main problem for EG analysis with liquid chromatography-tandem mass spectrometry (LC–MS/MS) is its chemical structure, with a molecular mass of 62 Da. Such small molecules are in general problematic for ion fragmentation. Another issue in the case of EG is a problematic electrospray (+ESI) ionisation. The application of analyte derivatisation seemed to be the only way to circumvent these problems [9].

Recent examinations revealed, that analyte adduct formation can be applied to solve different analytical problems concerning LC–MS/MS analysis. Not only the method sensitivity/specificity/detection could be improved in some applications but also bigger ion fragments could be generated for small molecules and as a consequence real MS^2/MS^3 mass transitions could be applied in MS^n detection of problematic substances [12–20]. Therefore, the aim of this work was to examine if the analyte adduct formation/fragmentation strategy can be applied for a direct EG analysis with LC–MS/MS, on the basis of protein precipitation with the components of the mobile phase as the only sample preparation step. Since preliminary studies revealed (data not presented), that the negative ESI mode cannot be applied for these purposes (like for other problematic analytes examined), the focus was on the EG adduct identification/application in the positive ESI mode.

E-mail addresses: analytiker@chemist.com, Dziadosz.Marek@mh-hannover.de.

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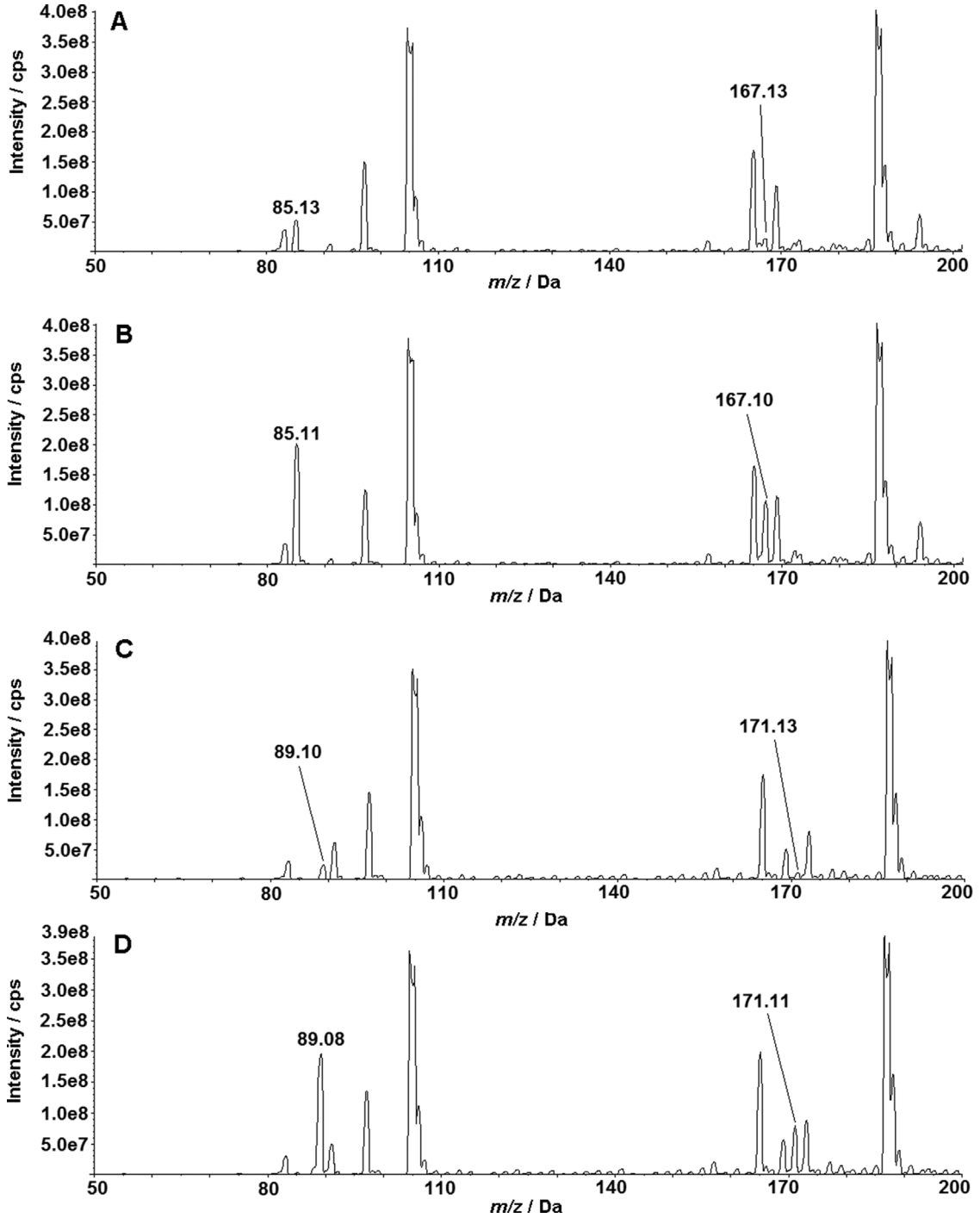

Fig. 1. +Q1 MS experiments performed with the following infusion solutions: A- 10 µg/mL EG, B- 100 µg/mL EG, G- 10 µg/mL EG-D4, D- 100 µg/mL EG-D4.

2. Materials and methods

The experimental part was performed with an Applied Biosystems API 4000 QTrap (AB Sciex Germany GmbH, Darmstadt) tandem mass spectrometer controlled by the Analyst 1.5 software and with a Shimadzu UFLC Prominence System (Shimadzu Deutschland GmbH, Duisburg) equipped with a Luna 5 µm C18 (2) 100 A, 150 mm × 2 mm analytical column (Phenomenex, Aschaffenburg, Germany). A 100 mg/mL EG (Sigma-Aldrich Chemie GmbH, Taufkirchen) stock solution was prepared in methanol and used for the preparation of appropriate analyte dilutions. A 10 mg/mL EG-D4 (Sigma-Aldrich Chemie GmbH, Taufkirchen) solution was used as internal standard (ISTD). Chemicals/solvents applied were of analytical/LC–MS grade and purchased from: Merck (Darmstadt, Germany) and J.T. Baker (Deventer, Netherlands). Blank human serum was purchased from the blood bank of the Hannover Medical School.

EG infusion solutions were prepared in methanol with 10 mmol L^{-1}/100 mmol L^{-1} CH_3COONa/CH_3COOH. A continuous infusion at a flow rate of 10 µL/min made the adduct identification/fragmentation experiments on the basis of the positive scan mode (+Q1 MS) and the positive enhanced product ion mode (+EPI) possible. For EG adduct identification/fragmentation different constellations of MS parameters were applied. For the purposes of this paper only one of them was described. Therefore, the source/gas parameters were as follows: curtain gas (N_2) − 10 psi, temperature − 0 (not controlled), ion source gas 1 (N_2) − 17 psi, ion source gas 2 (N_2) − 0 psi, ion spray